

### **REMARKS**

Claim 7-16 have been canceled without prejudice. Claims 1-6 have been amended, and new claims 17 and 18 have been added. In particular, independent claim 1 has been amended to more clearly designate the steps in the method, which include (a) “culturing plant material...wherein the plant material is protonema tissue...,” and (b) “obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells” as supported by original claim 1, and on page 16, line 17, to page 20, line 27, of the application as originally filed. Claims 2-4 have been amended to improve grammar. In addition, claim 3 has been amended to delete the phrase “or functional fragments thereof.” The present amendment has no limiting affect on these claims. Claims 5 and 6 have been amended to depend upon claim 1.

In addition, new claims 17 and 18 have been added. New independent claim 17 recites a “method for the production of heterologous proteinaceous substances in plant material” wherein the “plant material is... protonema tissue ... selected from the group consisting of *Physcomitrella*” as supported on page 9, lines 18-24, of the specification as originally filed. New claim 18 depends upon claim 17 and further recites that the “protonema tissue is *Physcomitrella patens*” as supported on page 16, lines 16-24, of the specification as originally filed.

No new matter has been added to the present application by the amendment.

### **The Invention**

The present invention pertains broadly to a method for producing heterologous substances in plant material, specifically cultured protonema tissue, and obtaining heterologous substances produced by the protonema tissue from culture medium without disrupting producing

tissues or cells. In one method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in plant material tissue, is provided having the steps recited in claim 1.

In accordance with another method embodiment of the present invention, a method for the production of heterologous proteinaceous substances in protonema *Physcomitrella* tissue is provided having the steps recited in claim 17.

Various other embodiments in accordance with the present invention are recited in the dependent claims. All of the embodiments in accordance with the present invention provide a method for producing heterologous proteinaceous substances in plant material, whether protonema moss tissue or protonema liverwort tissue, wherein the proteinaceous substances produced by the protonema tissue is advantageously obtained without disrupting producing tissues or cells.

Persons skilled in the art would recognize that the present invention advantageously utilizes protonema tissue, wherein “protonema” is defined in the art as “the usually filamentous thalloid stage of the gametophyte in mosses and in some liverworts comparable to the prothallium in ferns” (See Webster’s new collegiate dictionary, 1977, p. 927).

The novelty of the present invention over the prior art relates to the step of “obtaining the heterologous proteinaceous substances...without disrupting producing tissues or cells” because persons skilled in the art would not have predicted that proteinaceous substances could be obtained from protonema tissue, i.e., mature plant tissue having cell walls, without disrupting the producing tissue or cells.

### **The Rejection**

Claims 4, 6, 8-10 and 14-16 stand objected to under 37 C.F.R. § 1.75(c) as being in improper dependent form.

Claims 1-16 stand rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. Claims 3, 4-10 and 13-16 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

Claims 1, 2, 4, 5 and 8-13 stand rejected under 35 U.S.C. § 102(b) as anticipated by Houba-Hérin et al. (Nichole Houba-Hérin et al., *Cytokinin oxidase for Zea mays: purification, cDNA cloning and expression in moss protoplasts*. 17 The Plant Journal 615, 615-626 (1999))(hereafter, the “Houba-Hérin article”).

Claims 1-5 and 7-13 stand rejected under 35 U.S.C. § 103(a) as unpatentable over the Houba-Hérin article in view of Reutter et al. (K. Reutter and R. Reski, *Production of a heterologous protein in bioreactor cultures of fully differentiated moss plants*. 2 Plant Tissue Culture and Biotechnology 142, 142-147 (1996))(hereafter, the “Reutter article”). Claims 1-16 also stand rejected under 35 U.S.C. § 103(a) as unpatentable over the Houba-Hérin article in view of Nasu et al. (M. Nasu et al., *Efficient transformation of Marchantia polymorpha that is haploid and has very small genome DNA*. 84 J. Ferm. Bioengin. 519, 519-523 (1997))(hereafter, the “Nasu article”). Claims 1-16 also stand rejected under 35 U.S.C. § 103(a) as unpatentable over Zeidler et al. (M. Zeidler et al., *Transgene expression in the moss Ceratodon purpureus*. 154 J. Plant Physiol. 641, 641-650 (1999))(hereafter, the “Zeidler article”).

In view of the present amendment, Applicants respectfully traverse the rejection and request reconsideration for the following reasons.

**Applicants' Arguments**

In view of the present amendment, all claims 1-6, 17 and 18 are now in compliance with 37 C.F.R. § 1.75(c) and with 35 U.S.C. 112, second paragraph.

**The Examiner's Rejection Under 35 U.S.C. § 112, First Paragraph**

The Examiner admits that the present application is enabling for a method for the production of proteins in *Physcomitrella patens* (Office Action dated October 4, 2004, p. 2, lines 19-20), which is a bryophyte. For this reason, the Examiner's enablement rejection cannot apply to new claims 17 and 18.

However, the Examiner asserts that no other bryophytes are reasonably enabled by the present disclosure on the grounds that application of the heterologous gene in accordance with the present invention to any other bryophyte would require undue trial and error experimentation (Office Action dated October 4, 2004, p. 3, lines 10-13). On the other hand, the Examiner admits that (i) "Zeidler et al. disclose transformation of the moss *Ceratodon purpureus* (pg 643-647)" (October 4<sup>th</sup> Office Action, p. 6, lines 17-19) and also that (ii) "Nasu et al. teach transformation of *Marchantia polymorpha* (pg 520, left column, paragraphs 1-2)," which is a liverwort (October 4<sup>th</sup> Office Action, p. 6, lines 1-6).

Applicants contend that the Examiner's arguments are contradictory. Specifically, the Examiner admits that the present invention is enabled for *Physcomitrella patens* and then points out based on the prior art that a person of ordinary skill in the art would know how to genetically transform additional moss species (i.e., *Ceratodon purpureus*) and additional liverwort species (i.e., *Marchantia polymorpha*). The Examiner also argues that it would be obvious to do so on the grounds that "substitution of one bryophyte for another is an obvious optimization of design parameter" (October 4<sup>th</sup> Office Action, p. 6, lines 10-12). Applicants contend that the Examiner

has established, based on the prior art, that the transformation of mosses and liverworts is well known and predictable.

In view of these facts admitted by the Examiner, Applicants traverse the Examiner's grounds for the present lack of enablement rejection under 35 U.S.C. § 112 standing against the instant claims because the Examiner has produced no evidence grounded in the prior art in support of this rejection, and has evinced no sustainable reason for the rejection. In fact, the evidence adduced by the Examiner based on the prior art of record actually supports the conclusion that the present invention is enabled for both mosses and liverworts. The Examiner has simply failed to establish a prima facie case of lack of enablement under 35 U.S.C. § 112 as a matter of law.

### **The Elements of Enablement**

The statutory enablement requirement of 35 U.S.C. § 112, first paragraph, is a question of law pertaining to whether a specification teaches those of ordinary skill in the art how to make and use the full scope of the claimed invention without undue experimentation. In re Wright, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993). The initial burden rests on the Examiner to provide sufficient reasons for doubting assertions in the specification as to the scope of enablement. Id.

In the present case, the Examiner admits the specification is enabling to those of ordinary skill in the art for the bryophyte *Physcomitrella patens*, and the Examiner has produced evidence that a person skilled in the art would know how to transform *Ceratodon purpureus* and *Marchantia polymorpha* without undue experimentation. Surprisingly, the Examiner asserts that the heterologous gene inserted into a single species of bryophyte would not enable an ordinary person skilled in the art to practice the claimed invention without undue experimentation in any other bryophytes, not even closely related mosses such as *Funaria*, *Sphagnum*, *Ceratodon* and

other *Physcomitrella* species (October 4<sup>th</sup> Office Action, p. 3, lines 3-13). The Examiner's argument necessarily fails in view of the Examiner's admission that it is well known in the art to transform mosses, such as *Ceratodon*, and liverworts, such as *Marchantia*.

The Federal Courts have ruled that every species in a genus encompassed by the claims need not be disclosed in order to meet the enablement requirement for the genus; however, the enablement analysis must be made on a case-by-case basis. *In re Angstadt*, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976). Enablement is not precluded by the necessity for some experimentation; rather, the question is whether the amount of experimentation is "undue experimentation" as determined upon the weighing of many factors. *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). Factors to consider when determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *Id.*

The Examiner contends that the specification does not enable the claimed genus (in the patent law sense) because the amount of experimentation required to transform other mosses and liverworts would amount to "undue trial and error experimentation." (Office Action, dated October 4, 2004, page 3, lines 10-13). Applicants disagree for the following totality of reasons.

#### **Analysis of the Wands Factors for Claims Reciting the Protonema Tissue**

Claim 1 recites a "method for the production of heterologous proteinaceous substances in plant material...wherein the plant material is protonema tissue..." An analysis of the Wands Factors for methods for the production of heterologous proteinaceous substances in plant material in accordance with claims 1-6 is as follows.

### Direction and Guidance from Specification

First, the present specification gives ample guidance regarding how to practice and use the claimed invention as recited in claims 1-6. Specifically, the specification provides ample guidance regarding how to use intact plants to obtain heterologous proteinaceous substances directly from the culture medium without disrupting producing tissues or cells (See specification, page 9, line 5, to page 27, line 26). In other words, the present specification provides about 18 pages of detailed instruction.

Also, well-known and commercially available starting materials for practicing the methods are known in the prior art (see page 7, lines 1-8, and page 9, lines 6-24), with materials for a detailed example described on page 11, line 29, to page 26, line 18, of the present specification. In particular, the specification describes multiple species of mosses and liverworts that are well-known, characterized, and previously studied and that are suitable for practicing the present invention, including *Physcomitrella*, *Funaria*, and *Ceratodon* species (See specification, page 9, lines 6-24, and R. Reski, *Development, genetics and molecular biology of mosses*. 111 Bot. Acta 1, 1-15 (1998)(of record), hereafter the “Reski article”) as well as *Sphagnum* species (See specification, page 9, lines 6-18, and H. Rudolph and S. Rasmussen, *Studies on secondary metabolism of Sphagnum cultivated bioreactors*. 3 Crypt. Bot. 67, 67-73 (1992)(of record), hereafter the “Rasmussen article”).

Furthermore, the specification gives detailed direction and guidance regarding the genetic transformation of a single protonema species *Physcomitrella patens* so that the protonema tissue produces vascular endothelial growth factor VEGF (See specification as originally filed, page 11, line 29, to page 18, line 35). The specification explains that genetic transformation systems for *Physcomitrella patens* have been previously developed and described for enzyme production

(See specification, page 9, line 26, to page 10, line 2, and K. Reutter and R. Reski, *Production of heterologous protein in bioreactor cultures of fully differentiated moss plants*. 2 Plant Tissue Culture and Biotechnology 142, 142-147 (1996)(of record)) and for producing cytokines (See N. Houba-Herlin et al., *Cytokinin oxidase from Zea mays: purification, cDNA cloning and expression in moss protoplasts*. 17 The Plant Journal 615, 615-626 (1999)(of record)).

In view of the above facts demonstrating the application of gene transformation techniques to multiple species of plant protonema tissue, it is evident that a person of ordinary skill in the art would only have to apply routine experimentation to adapt the method described by the specific example provided by the instant specification, wherein the plant protonema tissue is *Physcomitrella patens*, to other species of protonema tissue whether mosses or liverworts.

#### Working Example Present

Second, the present specification provides a specific enabling example in the disclosure to teach how to perform and use the “method for the production of heterologous proteinaceous substances in plant material... wherein the plant material is protonema tissue” as described on page 11, line 29, to page 18, line 35 of the specification as originally filed. Protonema tissue is a well known plant tissue type, which manifests numerous common biological characteristics and functions whether originating from moss or liverwort species.

#### Nature of the Invention and State of the Art

Third, the present invention is directed to a method of producing heterologous proteinaceous substances using genetically transformed protonema tissue. Thus, the methods in accordance with this embodiment of the present invention employs simple plant organisms that have a well characterized plant physiology and predictable developmental cycle (See R. Reski,



*Development, genetics and molecular biology of mosses*. 111 Bot. Acta 1, 3, 6 and 11 (1998); and the Nasu article, p. 519, first column, lines 1-24). In other words, the biological plant organisms producing protonema tissue, whether of the moss phylum (e.g., *Physcomitrella*, *Funaria*, *Sphagnum* and *Ceratodon*) or the liverwort phylum (e.g., *Marchantia* and *Sphaerocarpos*), are relatively simple, predicatable organisms. The State of the Art is mature as evident from such prior art references as the Houba-Hérin article, the Reutter article, the Zeidler article, the Nasu article, the Rasmussen article, and the review article by H. Mühlbach, *Use of plant cell cultures in biotechnology*. 4 Biotechnology Annual Review 113, 158-161 (1998)(specifically, page 158, line 32, to page 161, line 6 (of record))(hereafter, the Mühlbach article). In fact, the Mühlbach article states that

[t]hese studies document the advanced stage that is currently achieved in the genetic transformation of *P. patens*, which can be certainly extended to other genes and also to other bryophytes with potential use in biotechnology.

Evidence along this line comes from studies on the expression of the human vascular endothelial growth factor (VEGF) protein in bioreactor cultures of *P. patens*....In general, these promising approaches clearly demonstrate the feasibility of bioreactor cultures of transgenic mosses for the production of heterologous compounds.

H. Mühlbach, at page 160, line 38, to page 161, line 6, (emphasis added).

In addition, the primary references recited against the claims of the present application, such as Houba-Hérin article, the Reutter article, the Zeidler article and the Nasu article, were published about 5 or more years ago, which further suggests the mature nature of the relevant art.

#### The Relative Skill of Those in the Art

Fourth, as is generally known, persons of ordinary skill in the art of transforming plant cells to express selected proteins, such as human VEGF, are highly trained professionals with

advanced degrees in cellular and molecular biology who are involved in research and technological advancement of the field. The relative skill level of those in the art is notably high.

#### Predictability or Unpredictability of the Art

Fifth, Applicants can point the Examiner back to the Zeidler article and the Nasu article to show that the transformation of other protonema species, such as *Ceratodon* and *Marchantia*, are known. The predictability of the art, pertaining to the transformation of plant species that ultimately produce protonema tissue, is highly predictable.

#### Quantity of Experimentation Necessary

Sixth, the present specification generally outlines the method for the production of heterologous proteinaceous substances in protonema tissue in accordance with claims 1-6 of the present invention, as described on page 8, line 1, to page 18, line 35, using known and readily available materials. The crux of the present invention is obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The method in accordance with these directions may require some quantity of experimentation in order to optimize results using other protonema species other than *Physcomitrella patens*, *Ceratodon purpureus* and *Marchantia polymorpha*; however, Applicants assert that while some experimentation may be necessary, it is no more than is commonly encountered in the art.

A person of ordinary skill in the art would know how to transform *Physcomitrella patens*, *Ceratodon purpureus* and *Marchantia polymorpha* protoplasts in view of Applicants' disclosure and the state of the art, without undue experimentation. However, transformation of plant protoplasts is not the crux of the invention. Be that as it may, transformation of other plant

protoplasts of species that produce protonema tissue is a matter of routine experimentation in the art in view of the biological predictability of protonema forming plant tissues in general.

#### Breadth of Claims

Seventh, the breadth of claim 1 includes the method of production of heterologous proteinaceous substances in protonema tissue. The term “protonema” has a specific meaning in the art and limits the scope of the present invention to tissues that are “the primary usually filamentous thalloid stage of the gametophyte in mosses and in some liverworts.” Furthermore, the claimed method includes two steps: (i) culturing protonema tissue in a culture medium to produce heterologous proteinaceous substances, and (ii) obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells. The breadth of claim 1 includes the species embodiment disclosed on page 11, line 29, to page 27, line 26. The breadth of claim 1 is not overly broad.

#### Summary of the Wands Factors Applied to the Protonema Tissue Embodiment

The above factors favor, as a matter of law, that Applicants’ application as originally filed would teach a person of ordinary skill in the art how to make and use the claimed invention without undue experimentation. Specifically, Applicants’ application provides (i) considerable direction and guidance on how to practice the invention, (ii) at least one very detailed working example of protonema tissue producing heterologous proteinaceous substances, (iii) there is an extremely high level of skill in the art, and (iv) the methods and materials needed to practice the invention are well known. While the degree of predictability in practicing the invention is not 100% (i.e., because not every possible protonema species has been studied in the prior art), multiple species of mosses and liverworts have been transformed in the prior art and by the

disclosure of the present invention. Therefore, the predictability is reasonably on par with, or more predictable than, other biotechnological arts that have been deemed enabled under similar circumstances. In re Wands, 8 U.S.P.Q.2d at 1406. Lastly, the breadth of the independent claim 1 of the present invention covers, in scope, the genus (in the patent law sense) of protonema tissue taught in the specification. The breadth of claim 1 is not overbroad.

### **The Examiner's Rejection Under 35 U.S.C. § 102(b)**

Anticipation under 35 U.S.C. § 102 requires showing the presence in a single prior art reference disclosure of each and every element of the claimed invention, arranged as in the claim. Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick, 221 U.S.P.Q. 481, 485 (Fed. Cir. 1984).

In the present case, the Houba-Hérin article teaches transforming moss protoplast cells, in particular *Physcomitrella patens* protoplasts, to express cytokinin oxidase (CKO) or  $\beta$ -glucuronidase (GUS) activity (Houba-Hérin article, See Abstract, and page 619, col. 2, lines 21-48, and page 624, col. 2, lines 35-53). However, the present invention, as recited in independent claims 1 and 20, pertains to obtaining heterologous proteinaceous substances from protonema tissue.

In other words, the Houba-Hérin article teaches transforming protoplasts, which a person skilled in the art would recognize are undifferentiated cells without cell walls (See Webster's new collegiate dictionary 927 (1977), "**protoplast...2 a** : the nucleus, cytoplasm, and plasma membrane of a cell constituting a living unit distinct from inert walls and inclusions"). On the other hand, the present invention obtains heterologous proteinaceous substances from "protonema tissue" as recited in claims 1 and 17, which a person skilled in the art would recognize are differentiated plant tissues (See Webster's new collegiate dictionary 927 (1977)).

“protonema...: the primary...thalloid stage of the gametophyte in mosses”). A person of ordinary skill in the art would know that such protonema tissue has cell walls.

While the present invention may make use of plant protoplasts when transforming cells, the present invention uses protonema tissue for producing the heterologous proteinaceous substances, and it is the heterologous proteinaceous substances produced by the protonema tissue that are obtained without disrupting producing protonema tissue or cells. Thus, while plant protoplasts may be used in preparing transformed protonema tissue for practicing the presently claimed invention, it is protonema tissue that is used in accordance with the present invention for producing the heterologous proteinaceous substances. Thus, plant protoplasts are not elements of the present invention as claimed.

Consequently, because the Houba-Hérin article does not teach, or even suggest, utilizing “protonema tissue” as recited in claims 1 and 17, in accordance with the present invention, the rejection under Section 102 is plainly untenable and must be withdrawn.

Applicants further point out that a person skilled in the art would not have predicted that using protonema tissue would be a good approach for easily obtaining heterologous proteinaceous substances from the medium for the reason that the producing protonema tissue comprises cells having cell walls. Typically, proteinaceous substances made by fully differentiated plants are trapped in a space located between the plasma membrane and the cell wall. Consequently, conventional retrieval of such substances from mature plants requires disrupting their cell wall, e.g. by mechanical or chemical means.

It is thus evident that the Houba-Hérin article does not disclose or suggest the step, in accordance with claim 1 of the present invention, of obtaining heterologous proteinaceous substances from culture medium of protonema tissue without disrupting producing protonema tissue or cells.

### **The Examiner's Rejection Under 35 U.S.C. § 103(a)**

A patentability analysis under 35 U.S.C. § 103 requires (a) determining the scope and content of the prior art, (b) ascertaining the differences between the prior art and the claimed subject matter, (c) resolving the level of ordinary skill in the pertinent art, and (d) considering secondary considerations that may serve as indicia of nonobviousness or obviousness. Graham v. John Deere Co. of Kansas City, 148 U.S.P.Q. 459, 467 (1966). Furthermore, a proper rejection under Section 103 further requires showing (1) that the prior art would have suggested to a person of ordinary skill in the art that they should make the claimed device or carry out the claimed process, (2) that the prior art would have revealed to a person of ordinary skill in the art that in so making or doing, there would have been a reasonable expectation of success, and (3) both the suggestion and the reasonable expectation of success must be found in the prior art and not in the applicants' disclosure. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991).

The Examiner's Section 103 rejection is untenable for the following reasons.

### **The Houba-Hérin article**

The Houba-Hérin article, as characterized above, pertains to transforming and using moss protoplast cells, in particular *Physcomitrella patens* protoplasts, to express cytokinin oxidase (CKO) or  $\beta$ -glucuronidase (GUS) activity. The Houba-Hérin article teaches culturing single protoplasts and not differentiated plant tissue (Houba-Hérin article at 624, second column, lines 35-54). Thus, the Houba-Hérin article is completely silent with respect to culturing protonema tissue. Furthermore, because the Houba-Hérin article does not address culturing plant tissue comprising cells having cell walls, the Houba-Hérin article cannot reasonably address the problems faced in recovering expressed heterologous proteins from differentiated plant tissue

(which has cell walls) “without disrupting producing tissues or cells” as recited in independent claims 1 and 17.

#### The Reutter Article

The Reutter article teaches the transformation of moss protoplasts, in particular *Physcomitrella patens* protoplasts, using the PEG-mediated direct DNA transfer with a 11.5 kb plasmid carrying the *E. coli gus*-gene under the control of the cauliflower mosaic virus (CaMV) 35S-promoter as well as the *nptII*-gene under control of the *Agrobacterium tumefaciens* nopal synthase promoter (Reutter article, page 143, line 4, to page 144, line 5). The Reutter article also discloses culturing protonemata to form protonema balls using high speed stirring (Reutter article, at 145, lines 1-5). However, the Reutter article is completely silent with respect to obtaining the  $\beta$ -glucuronidase produced by the moss protonema balls without disrupting producing tissues or cells as recited in claims 1 and 17.

#### The Zeidler Article

The Zeidler article pertains to the transgene expression in the moss *Ceratodon purpureus* of certain reporter genes such as screenable GUS, LUC and GFP reporters, the expression products of which are located intracellularly (See Abstract). In particular, the Zeidler article teaches that moss protoplasts or filaments may be investigated using a microscope and staining, or by assaying protoplast cultures following destruction of cells by either use of a French press, or by grinding with sand (Zeidler article at 643, first col., lines 3-30). Persons of ordinary skill in the art would realize that cellular staining techniques, such as used by the Zeidler article, are generally toxic to cells and would disrupt producing tissues or cells. In addition, it is plain that the techniques taught by the Zeidler article for assaying the supernatant of incubated cells

requires destroying the cells. Therefore, the Zeidler article does not teach, or even suggest, the step of “obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells” as recited in claims 1 and 17 of the present application.

#### The Mühlbach Article

The review article titled “Use of plant cell cultures in biotechnology” by Hans-Peter Mühlbach, *Biotechnology Annual Review*, 1998, pp. 113-176 (of record, and hereafter referred to as the “Mühlbach article”) pertains to the use of plant cell cultures in biotechnology. This article discusses a “personal communication” regarding studies on the expression of the human vascular endothelial growth factor (VEGF) protein in bioreactor cultures of *Physcomitrella patens* moss (Mühlbach article at 160, lines 38-40). The author writes that

“*Physcomitrella* protoplasts were efficiently transformed with constructs harbouring the VEGF coding sequence under the control of constructive as well as inducible promoters, and transgenic moss plants were grown in the bioreactor described above. Expression analysis of this interesting heterologous protein is awaited in the near future.”

Mühlbach article, page 160, line 42, to page 161, line 4.

Nothing is said about obtaining VEGF from the culture medium without disrupting producing tissues or cells. In fact, Applicants assert that the studies referred to in the Mühlbach article involved disrupting the producing cells before detecting VEGF in culture medium.

In summary, the Mühlbach article does not teach, or even suggest, the step of “obtaining the proteinaceous substances from the culture medium without disrupting producing tissues or cells” as recited in claims 1 and 17 of the present application. The Mühlbach article merely reports investigator success at transforming *Physcomitrella* protoplasts with a VEGF coding sequence; however, the report explicitly does not address expression analysis (Mühlbach article, at 161, lines 3-4).



### The Nasu Article

The Nasu article pertains to the transformation of *Marchantia polymorpha* that is a haploid liverwort with very small genome DNA (See Abstract). In particular, the Nasu article teaches transforming single *Marchantia* cells with a binary vector plasmid pB1121 and a plasmid pRiA4b so as to inactivate the GUS gene (Nasu article, at 520, first column, lines 1-11). Thus, the Nasu article pertains to cultures of single cells, and not to intact plant tissue. Furthermore, transformed cells were fixed in formaldehyde, which a person of ordinary skill in the art would realize kills the cells, and then the dead cells were stained for GUS activity (Nasu article, at 520, first column, lines 35-47, and see Figure 4). The Nasu article plainly does not teach, or even suggest, the step of “obtaining the proteinaceous substances from the culture medium without disrupting producing tissues or cells” as recited in claims 1 and 17 of the present application.

### Summary of the Prior Art

In summary, the Houba-Hérin article teaches transforming and using *Physcomitrella patens* protoplasts to express cytokinin oxidase (CKO) or  $\beta$ -glucuronidase (GUS) activity, but it does not teach culturing protonema tissue. The Reutter article teaches the transformation of *Physcomitrella patens* protoplasts using the PEG-mediated direct DNA transfer in order to intracellularly accumulate  $\beta$ -glucuronidase. While the Reutter article teaches culturing moss protonemata and protonema balls, the article is completely silent with respect to obtaining the  $\beta$ -glucuronidase produced by the protonema without disrupting producing tissues or cells. The Zeidler article teaches transgene expression in the moss *Ceratodon purpureus* of certain reporter genes such as screenable GUS, LUC and GFP reporters, the expression products of which are located intracellularly, wherein moss protoplasts or filaments may be investigated using a microscope and toxic staining, or by assaying macerated protoplast cultures. The Nasu article

teaches transforming *Marchantia* cells with a binary vector plasmid pB1121 and a plasmid pRiA4b so as to inactivate the GUS gene followed by fixing in formaldehyde and staining for GUS activity.

Plainly, none of these prior art references reasonably teach, or even suggest, the steps of “culturing ...protonema tissue” and “obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells” as recited in claims 1 and 17 of the present application. For this reason alone, the Examiner’s Section 103 rejection is untenable and must be withdrawn.

### **Conclusion**

Claims 1-6, 17 and 18 are now in compliance with 35 U.S.C. § 112, second paragraph. Claims 17 and 18 are enabled for the reasons conceded by the Examiner. Claims 1-6 pertain to a method for the production of heterologous proteinaceous substances in protonema tissue, and complies with the enablement requirement of 35 U.S.C. § 112, first paragraph, for the reasons discussed above.

The rejection under 35 U.S.C. § 102(b) is untenable and must be withdrawn because the Houba-Hérin article teaches transforming and culturing moss protoplasts, and does not pertain to “culturing...protonema tissue” as recited in claims 1 and 17 of the present application. Furthermore, the Houba-Hérin article does not teach, or even suggest, the step of “culturing...protonema tissue” and “obtaining the heterologous proteinaceous substances from the culture medium without disrupting producing tissues or cells” as recited in claims 1 and 17 because the Houba-Hérin article does not pertain to protonema tissue.


The rejection under 35 U.S.C. § 103(a) is untenable and must be withdrawn because none of the prior art references teach, or even suggest, the steps of “culturing...protonema tissue” and “obtaining the proteinaceous substances from the culture medium without disrupting producing tissues or cells” as recited in independent claims 1 and 17 of the present application.

For all of the above reasons, claims 1-6, 17 and 18 are in condition for allowance, and a prompt notice of allowance is earnestly solicited.

Questions are welcomed by the below-signed attorney for applicants.

Respectfully submitted,

GRIFFIN & SZIPL, PC

  
 \_\_\_\_\_  
 Joerg-Uwe Szimpl  
 Reg. No. 31,799

GRIFFIN & SZIPL, PC  
 Suite PH-1  
 2300 Ninth Street, South  
 Arlington, VA 22204

Telephone: (703) 979-5700  
 Facsimile: (703) 979-7429  
 Customer No.: 24203

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**protestant** *adj* 1 *cap* : of or relating to Protestants, their churches, or their religion 2 : making or sounding a protest <the two ~ ladies up and marched out — Time>

**protes-tation** \prōt-əs-'tā-shən, -prōt-ēs-, -prōt-əs-, -prōt-es-\ *n* : the act of protesting : a solemn declaration or avowal

**pro-te-us** \prōt-ē-əs\ *n*, *pl* -tei -ē-i\ [NL, genus name, fr. L. Proteus] : any of a genus (*Proteus*) of aerobic gram-negative usu. motile bacteria that include saprophytes in decaying organic matter and forms associated with gastrointestinal disorders

**Pro-te-us** \prōt-(y)ūs, -prōt-ē-əs\ *n* [L. fr. Gk *Prōteus*] : a Greek sea god capable of assuming different forms

**pro-tha-la-mi-on** \prō-thā-'lā-mē-ən, -'lān\ or **pro-tha-la-mi-um** \-mē-əm\ *n*, *pl* -mīa -mē-ə\ [NL, fr. Gk *pro-* + *-thalamion* (as in *epithalamion*)] : a song in celebration of a marriage

**pro-thal-li-um** \prō-thāl-'ē-əm\ *n*, *pl* -thāl-līa -ē-ə\ [NL, fr. *pro-* + *thallus*] 1 : the gametophyte of a pteridophyte (as a fern) that is typically a small flat green thallus attached to the soil by rhizoids 2 : a greatly reduced structure of a seed plant corresponding to the pteridophyte prothallium — **pro-thal-li-al** \-ē-əl\ *adj*

**pro-thal-lus** \prō-thāl-'as\ *n* [NL, fr. *pro-* + *thallus*] : PROTHALLIUM

**pro-the-sis** \prōth-ə-'sēs\ *n*, *pl* -ēs -sēs\ [LL, alter. of *prothesis*, fr. Gk, lit., addition — more at PROSTHESIS] : the addition of a sound to the beginning of a word (as in Old French *estat* — whence English *estate* — from Latin *status*) — **pro-thet-ic** \prō-thet-'ik\ *adj*

**prothe-tely** \prōth-ə-'tel-ē\ *n* [perh. fr. Gk *prothēnai* to put before (fr. *pro-* + *thēnai* to put) + *telein* to complete, perfect, fr. *telos* end — more at DO, WHEEL] : relatively precocious differentiation of a structure usu. associated with a later stage of development — **prothe-tic** \prōth-ə-'tel-'ik\ *adj*

**pro-tho-no-ta-ry** \prō-thān-ə-'ter-ē, -prō-thān-ōt-ə-rē\ or **pro-tho-no-ta-ry** \prō-thān-ə-'ter-ē, -prōt-ə-nōt-ə-rē\ *n*, *pl* -ries [ME *prothotary*, fr. LL *prothotarius*, fr. *pro-* + L *notarius* notary] : a chief clerk of any of various courts of law — **pro-tho-no-tar-i-al** \prō-thān-ə-'ter-ē-əl, -prō-thān-ōt-er-ē-əl\ *adj*

**pro-tho-rac-ic** \prō-thā-'ras-'ik\ *adj* : of or relating to the thorax

**prothoracic gland** *n* : one of a pair of thoracic endocrine organs in some insects that control molting

**pro-tho-rax** \prō-thō-'raks, -'thō-ə-r-\ *n* [NL *prothorax*, *prothorax*, fr. *pro-* + *thorax*] : the anterior segment of the thorax of an insect — see INSECT illustration

**pro-throm-bin** \prō-'thrām-bən\ *n* [ISV] : a plasma protein produced in the liver in the presence of vitamin K and converted into thrombin in the clotting of blood

**pro-tist** \prōt-'st, -'stis\ *n* [deriv. of Gk *prōtistos* very first, primal, fr. *superl.* of *protos* first — more at PROT-] : any of a kingdom or other group (Protista) of unicellular or acellular organisms comprising bacteria, protozoans, various algae and fungi, and sometimes viruses — **pro-tis-tan** \prō-'tis-tən\ *adj* or *n* **pro-ti-um** \prōt-ē-əm, -prō-shē-\ *n* [NL, fr. Gk *protos* first] : the ordinary light hydrogen isotope of atomic mass 1

**proto-** — see PROT-

**pro-to-col** \prōt-ə-'kōl, -'kōl, -'kāl, -'kəl\ *n* [MF *prothocolle*, fr. ML *protocolum*, fr. LGK *protokollon* first sheet of a papyrus roll bearing data of manufacture, fr. Gk *prōt-* + *kollan* to glue together, fr. *kolla* glue; akin to MD *helen* to glue] 1 : an original draft, minute, or record of a document or transaction 2 a : a preliminary memorandum often formulated and signed by diplomatic negotiators as a basis for a final convention or treaty b : the records or minutes of a diplomatic conference or congress that show officially the agreements arrived at by the negotiators 3 : a code prescribing strict adherence to correct etiquette and precedence (as in diplomatic exchange and in the military services) 4 : the plan of a scientific experiment or treatment

**pro-to-derm** \prōt-ə-'därm\ *n* [ISV] : DERMATOGEN — **pro-to-der-mal** \prōt-ə-'dər-məl\ *adj*

**pro-to-gal-axy** \prōt-ə-'gal-ək-sē\ *n* : a hypothetical cloud of gas believed to have condensed into stars and formed the galaxies

**pro-to-his-to-ry** \prōt-'hīst-ō-rē\ *n* [ISV] : the study of man in the times that immediately antedate recorded history — **pro-to-his-to-ri-an** \prōt-'hīst-ō-rē-ən, -'tōr-\ *n* — **pro-to-his-tor-ic** \-tōr-'ik, -'tār-\ *adj*

**pro-to-hu-man** \prōt-'hyū-mən, -'yū-\ *adj* : of, relating to, or resembling an early primitive human or a manlike primate — **protohu-man** *n*

**pro-to-lan-guage** \prōt-ə-'lāŋ-gwī-\ *n* : an assumed or recorded ancestral language

**pro-to-lith-ic** \prōt-ə-'lith-'ik\ *adj* : of or relating to the earliest period of the Stone Age : EOLITHIC

**pro-to-mar-tyr** \prōt-ə-'mārt-ər-\ *n* [ME *prothomartir*, fr. MF, fr. LL *protomartyr*, fr. LGK *protomartyr*, *protomartyr*, fr. Gk *prōt-* + *martyr*, *martyr* martyr] : the first martyr in a cause or region

**pro-ton** \prōt-'tān\ *n* [Gk *prōton*, neut. of *prōtos* first — more at PROT-] : an elementary particle that is identical with the nucleus of the hydrogen atom, that along with neutrons is a constituent of all other atomic nuclei, that carries a positive charge numerically equal to the charge of an electron, and that has a mass of  $1.672 \times 10^{-24}$  gram — **pro-ton-ic** \prōt-'tān-'ik\ *adj*

**pro-ton-ate** \prōt-ə-'nāt\ *vb* -at-ed; -at-ing *vi* : to add a proton to ~ *vi* : to acquire an additional proton — **pro-ton-ation** \prōt-ə-'nā-shən\ *n*

**pro-to-ne-ma** \prōt-ə-'nē-mə\ *n*, *pl* -nē-ma-tə -'nē-mət-ə, -'nem-ət-\ [NL *protonema*, *protonema*, fr. *pro-* + Gk *nēma* thread — more at NEMAT-] : the primary usu. filamentous thalloid stage of the gametophyte in mosses and in some liverworts comparable to the prothallium in ferns — **pro-to-ne-mal** \-nē-məl\ *adj* — **pro-to-ne-ma-tal** \-nē-mət-'l, -'nem-ət-\ *adj*

**protonotary apostolic** or **prothonotary apostolic** *n*, *pl* **protonotaries apostolic** or **prothonotaries apostolic** : a priest of the chief college of the papal curia who keeps records of consistories and canonizations and signs papal bulls; also : an honorary member of this college

**pro-ton-syn-chro-tron** \prō-'tān-'sīŋ-k(r)-ə-'trān, -'sīn-\ *n* : a synchrotron in which protons are accelerated by means of frequency modulation of the radio-frequency accelerating voltage so that they have energies of billions of electron volts

**pro-to-nymph** \prōt-ə-'nim(p)-\ *n* : any of various acarids in their first developmental stage — **pro-to-nymph-al** \prōt-ə-'nim(p)-fəl\ *adj*

**pro-to-path-ic** \prōt-ə-'path-'ik\ *adj* [ISV, fr. MGK *prōtopathēs* affected first, fr. Gk *prōt-* + *pathos* experience, suffering — more at PATHOS] : of, relating to, or being cutaneous sensory reception responsive only to rather gross stimuli

**pro-to-phlo-em** \prōt-ə-'flō-əm\ *n* : the first-formed phloem that develops from procambium, consists of narrow thin-walled cells capable of a limited amount of stretching, and is usu. associated with a region of rapid growth

**pro-to-plan-et** \prōt-ə-'plan-ət\ *n* : a hypothetical whirling gaseous mass within a giant cloud of gas and dust that rotates around a sun and is believed to give rise to a planet

**pro-to-plasm** \prōt-ə-'plaz-əm\ *n* [G *protoplasma*, fr. *pro-* + NL *plasma*] 1 : the organized colloidal complex of organic and inorganic substances (as proteins and water) that constitutes the living nucleus, cytoplasm, plastids, and mitochondria of the cell and is regarded as the only form of matter in which the vital phenomena are manifested 2 : CYTOPLASM — **pro-to-plas-mic** \prōt-ə-'plaz-'mīk\ *adj*

**pro-to-plast** \prōt-ə-'plast\ *n* [MF *protoplaste*, fr. LL *protoplastus* first man, fr. Gk *protoplastos* first formed, fr. *prōt-* + *plastos* formed, fr. *plassein* to mold — more at PLASTER] 1 : one that is formed first : PROTOTYPE 2 a : the nucleus, cytoplasm, and plasma membrane of a cell constituting a living unit distinct from inert walls and inclusions b : ENERGID — **pro-to-plas-tic** \prōt-ə-'plas-'tik\ *adj*

**pro-to-por-phy-rin** \prōt-ə-'pōr-f(-ə)-rən\ *n* [ISV] : a purple porphyrin acid  $C_{24}H_{14}N_4O_6$  obtained from hemin or heme by removal of bound iron

**pro-to-star** \prōt-ə-'stār\ *n* : a hypothetical cloud of dust and atoms in space believed to develop into a star

**pro-to-stele** \prōt-ə-'stēl, -prōt-ə-'stē-lē\ *n* : a stele forming a solid rod with the phloem surrounding the xylem — **pro-to-ste-lie** \prōt-ə-'stē-'lē\ *adj*

**pro-to-troph** \prōt-ə-'trōf, -'träf\ *n* [back-formation fr. *prototrophic*] : a prototrophic individual

**pro-to-tro-phic** \prōt-ə-'trōf-'ik\ *adj* [ISV] : deriving nutriment from inorganic sources — **pro-to-tro-phy** \prōt-ə-'trōf-ē\ *n*

**pro-to-typ-al** \prōt-ə-'ti-pəl\ *adj* : of, relating to, or constituting a prototype : ARCHETYPAL

**pro-to-type** \prōt-ə-'tīp\ *n* [F, fr. Gk *prototypon*, fr. neut. of *prōtotypos* archetypal, fr. *prōt-* + *typos* type] 1 : an original model on which something is patterned : ARCHETYPE 2 : an individual that exhibits the essential features of a later type 3 : a standard or typical example 4 : a first full-scale and usu. functional form of a new type or design of a construction (as an airplane)

**pro-to-typ-ic-al** \prōt-ə-'tīp-'i-kəl\ also **pro-to-typ-ic** \-ik\ *adj* : PROTOTYPAL — **pro-to-typ-i-cal-ly** \-i-kəl\ *adv*

**pro-to-xylem** \prōt-ə-'zīl-əm, -'dēm\ *n* : the first-formed xylem developing from procambium and consisting of narrow cells with annular, spiral, or scalariform wall thickenings

**pro-to-zo-al** \prōt-ə-'zō-əl\ *adj* : of or relating to protozoans

**pro-to-zo-an** \prōt-ə-'zō-ən\ *n* [NL *Protozoa*, phylum name, fr. *pro-* + *-zoa*] : any of a phylum or subkingdom (Protozoa) of minute protoplasmic acellular or unicellular animals which have varied morphology and physiology and often complex life cycles, which are represented in almost every kind of habitat, and some of which are serious parasites of man and domestic animals — **protozoan** *adj* — **pro-to-zo-ic** \-zō-'ik\ *adj*

**pro-to-zo-ol-o-gy** \prōt-ə-'zō-'āl-ə-'jē, -'zō-'wāl-\ *n* [NL *Protozoa* + ISV *-logy*] : a branch of zoology dealing with protozoans — **pro-to-zo-ol-o-gist** \prōt-ə-'zō-'āl-ə-'jēst, -'zō-'wāl-\ *n*

**pro-to-zo-on** \prōt-ə-'zō-ən\ *n*, *pl* -zoa -'zō-ə\ [NL, fr. sing. of *Protozoa*] : PROTOZOAN

**pro-tract** \prō-'trakt, p(r)-ə-\ *vi* [L *protractus*, pp. of *protrahere*, lit., to draw forward, fr. *pro-* forward + *trahere* to draw — more at PRO, DRAW] 1 *archaic* : DELAY, DEFER 2 : to prolong in time or space 3 : to lay down the lines and angles of with scale and protractor : PLOT 4 : to extend forward or outward *syn* see EXTEND *ant* curtail — **pro-trac-tive** \-trāk-'tīv\ *adj*

**protracted meeting** *n* : a revival meeting extending over a period of time

**pro-trac-tile** \-trāk-'tīl, -'tīl\ *adj* [L *protractus*] : capable of being thrust out <~ jaws>

**pro-trac-tion** \-trāk-'shən\ *n* [LL *protraction*, *protractio* act of drawing out, fr. *protractus*] 1 : the act of protracting : the state of being protracted 2 : the drawing to scale of an area of land

**pro-trac-tor** \-trāk-'tər\ *n* 1 a : one that protracts, prolongs, or delays b : a muscle that extends a part 2 : an instrument that is used for laying down and measuring angles in drawing and plotting

**pro-trep-tic** \prō-'trep-'tik\ *n* [LL *protrepticus* hortatory, encouraging, fr. Gk *protreptikos*, fr. *protrepein* to turn forward, urge on, fr. *pro-* + *trepein* to turn — more at TROPE] : an utterance (as a speech) designed to instruct and persuade — **pro-trep-tic** \-trep-'tik\ *adj*

**pro-trude** \prō-'trūd\ *vb* **pro-trud-ed**; **pro-trud-ing** [L *protrudere*, fr. *pro-* + *trudere* to thrust — more at THREAT] *vi* 1 *archaic* : to thrust forward 2 : to cause to project or stick out ~ *vi* : to jut out from the surrounding surface or context <~ a handker-

ə abut    ʰ kitten    ər further    ʰ back    ʰ bake    ʰ cot, cart  
aʊ out    ʰ chin    ɛ less    ɛ easy    ɡ gift    ɪ trip    ɪ life  
j joke    ɟ sing    ɔ flow    ɔ flaw    ɔl coin    ʰ thin    ʰ this  
ʊ loot    ʊ foot    ʏ yet    ʏ few    ʏ furious    ʒb vision

BEST AVAILABLE COPY

broce] 1: a device  
d esp. for sweeping,  
ing a brush; as a  
3 a: an electrical  
akes sliding contact  
erator or a motor  
b: a quick light

apply with a brush  
ush) <~ed the dirt  
ay: DISMISS <~ed  
uch gently against

r to dash through  
lessly <~ed by the

bruschen]: a brief

application with a

near the batter's  
e back from home

membrane of an  
is specialized for

ely slow electrical

~ fabric>  
volving brush but  
ly on a small and

rush growth  
l dismissal  
eliminating small  
to refresh one's  
th) — brush-up

all branches esp.

small trees

(as in painting);

brush

ROUGH

ounding in brush

brusco, fr. ML

and abrupt 2

of ungracious

brusque-ly adv

abruptness of

um]: a carpet

indation web of

form the pattern

compact rough

led also griffin

point or bobbin

Brussels 2: a

# BEST AVAILABLE COPY

bryophyte • bucktail

bryo-phyte \bri-ə-ˈfīt/ n [deriv. of Gk *bryon* + *phyton* plant; akin to Gk *phyein* to bring forth — more at BE]: any of a division (Bryophyta) of nonflowering plants comprising the mosses and liverworts — *bryo-phytic* \bri-ə-ˈfīt-ik/ adj

bryo-zo-an \bri-ə-ˈzō-ən/ n [NL *Bryozoa*, class name, fr. Gk *bryon* + NL *-zoa*]: any of a phylum or class (Bryozoa) of aquatic mostly marine invertebrate animals that reproduce by budding and usu. form permanently attached branched or mossy colonies — *bryo-zoan* adj

Bryth-on \brith-ˈän, -ən/ n 1: a member of the British branch of Celts 2: a speaker of a Brythonic language

Bry-thon-ic \brith-ˈän-ik/ adj 1: of, relating to, or characteristic of the Brythons 2: of, relating to, or characteristic of the division of the Celtic languages that includes Welsh, Cornish, and Breton

Brythonic n: the Brythonic branch of the Celtic languages — see INDO-EUROPEAN LANGUAGES table

BS abbr 1 bachelor of science 2 balance sheet 3 bill of sale 4 British standard

BSA abbr 1 bachelor of science in agriculture 2 Boy Scouts of America

BSAA abbr bachelor of science in applied arts

BSArch abbr bachelor of science in architecture

BSAE abbr 1 bachelor of science in aeronautical engineering 2

bachelor of science in agricultural engineering 3 bachelor of

science in architectural engineering

BSAg abbr bachelor of science in agriculture

BSB abbr bachelor of science in business

BSc abbr bachelor of science

BSCh abbr bachelor of science in chemistry

BSE or BSEcon abbr bachelor of science in economics

BSED or BSE abbr bachelor of science in education

BSEE abbr bachelor of science in elementary education

BSFor abbr bachelor of science in forestry

BSFS abbr bachelor of science in foreign service

BSI abbr British Standards Institution

bskt abbr basket

BSL abbr 1 bachelor of sacred literature 2 bachelor of science in

languages 3 bachelor of science in law 4 bachelor of science in

linguistics

BSN abbr bachelor of science in nursing

btry abbr battery

Btu abbr British thermal unit

bu abbr 1 bureau 2 bushel

'bub-bil \bub-əl/ vb bub-bled; bub-ling \bub-(ə-)lɪŋ/ [ME

*bublen* vi 1: to form or produce bubbles 2: to flow with a

gurgling sound <a brook bubbling over rocks> 3 a: to become

lively or effervescent <bubbling with good humor> b: to speak

in a lively and fluent manner <bubbled excitedly about his prize>

~ vi 1: to utter (as words) effervescently 2: to cause to bubble

'bubble n, often attrib 1: a small globule typically hollow and

light: as a: a small body of gas within a liquid b: a thin film

of liquid inflated with air or gas c: a globule in a transparent

solid d: something that is hemispherical or semicylindrical 2 a:

something that lacks firmness, solidity, or reality b: a delusive

scheme 3: a sound like that of bubbling

bubble and squeak n, chiefly Brit: a dish consisting of potatoes,

cabbage, and sometimes meat fried together

bubble chamber n: a chamber of heated liquid in which the path

of an ionizing particle is made visible by a string of vapor bubbles

bubble gum n 1: a chewing gum that can be blown into large

bubbles 2: rock music characterized by simple repetitive phras-

ings and intended esp. for young teenagers

bub-ble \bub-(ə-)lɪr/ n 1: one that bubbles 2: a drinking

fountain from which a stream of water bubbles upward

'bub-bly \bub-(ə-)li/ adj bubb-li-er; -est 1: full of bubbles

EFFERVESCENT <a ~ bottle of pop> 2: showing lively good

spirits <a ~ group at the celebration> 3: resembling a bubble <a

~ dome>

2'bubbly n: CHAMPAGNE

bubby var of BOOBY

bu-bo \b(y)ü-(b)ō/ n, pl buboes [ML *bubon*, *buba*, fr. Gk

*boubōn*]: an inflammatory swelling of a lymph gland esp. in the

groin — *bu-bon-ic* \b(y)ü-(b)ān-ik/ adj

bubonic plague n: plague in which the formation of buboes is a

prominent feature

buc-cal \bək-əl/ adj [L *bucca* cheek — more at POKK]: of, relating

to, or involving the cheeks or the cavity of the mouth

buc-ca-neer \bək-ə-ˈni(ə)r/ n [F *boucanier*] 1: one of the

freebooters preying on Spanish ships and settlements esp. in the

West Indies in the 17th century; broadly: PIRATE 2: an un-

scrupulous adventurer esp. in politics or business — *buccaneer* vi

— *buc-ca-neer-ish* \-ish/ adj

Bu-ceph-a-lus \byü-ˈsɛf-ə-ˈlās/ n [L, fr. Gk *Boukephalos*]: the

war-horse of Alexander the Great

'buck \bək/ n, pl bucks [ME, fr. OE *bucca* stag, he-goat; akin to

OHG *boc* he-goat, MLr *bocc*] 1 or pl buck: a male animal; esp.

a: a male deer or antelope 2 a: a male human being: MAN b

: a dashing fellow: DANDY 3 or pl buck: ANTELOPE 4 a

: BUCKSKIN; also: an article (as a shoe) made of buckskin b slang

DOLLAR 3b 5 [short for *sawbuck*]: SAWHORSE 8 a: a support-

ing rack or frame b: a short thick leather-covered block for

gymnastic vaulting

'buck vi 1 of a horse or mule: to spring with a quick plunging

leap 2: to charge against something (as an obstruction) 3 a:

to move or react jerkily b: to refuse assent: BALK 4: to

strive for advancement sometimes without regard to ethical

behavior ~ vi 1: to throw (as a rider) by bucking 2 a *archaic*

: BUTT b: OPPOSE, RESIST <~ing a trend> 3: to charge into (as

the opponent's line in football) 4 a: to pass esp. from one person

to another <~ed the question on to someone else> b: to move

or load (as heavy objects) esp. with mechanical equipment — *buck-*

er n

3'buck adj [prob. fr. 'buck]: of the lowest grade within a military

category <~ private>

'buck n [short for earlier *buckhorn knife*]: an object formerly used in poker to mark the next player to deal; broadly: a token used as a mark or reminder

'buck adv [origin unknown] South & Midland: STARK <~ naked>

buck-and-wing \bək-ən-ˈwɪŋ/ n: a solo tap dance with sharp foot accents, springs, leg flings, and heel clicks

buck-a-roo or buck-er-oo \bək-ə-ˈru:, ˈbək-ə-ˈru:/ n, pl -aroos or -eroos [by folk etymology fr. Sp *vaquera*, fr. *vaca* cow, fr. L *vacca* — more at VACCINE] 1: COWBOY 2: BRONCOBUSTER

buck-bean \bək-ˈben/ n: a plant (*Menyanthes trifoliata* of the family Menyanthaceae) growing in bogs and having racemes of white or purplish flowers

buck-board \-bō(ə)rd, -bō(ə)rd/ n [obs. E *buck* body of a wagon + E *board*]: a four-wheeled vehicle with a springy platform

'buck-et \bək-ət/ n [ME, fr. AF *buket*, fr. OE *buc* pitcher, belly; akin to OHG *buh* belly, Skt *bhūri* abundant — more at BIG] 1: a typically round vessel for catch-

ing, holding, or carrying liquids or solids 2: something resembling a bucket: as a: the scoop of an excavating machine b: one of the receptacles on the rim of a

waterwheel c: one of the cups of an endless-belt conveyor d

: one of the vanes of a turbine rotor 3: a large quantity 4

: BUCKET SEAT

2'bucket vt 1: to draw or lift in buckets 2 Brit a: to ride (a horse) hard b: to drive hurriedly or roughly 3: to deal with in

a bucket shop ~ vi 1: HUSTLE, HURRY 2 a: to move about haphazardly or irresponsibly b: to move roughly or jerkily

<~ing over the rocky road>

bucket brigade n: a chain of persons acting to put out a fire by

passing buckets of water from hand to hand

buck-et-ful \bək-ət-ˈfʊl/ n, pl bucketfuls \-fʊl/ or buck-et-ful \-ts-ˈfʊl/: as much as a bucket will hold

bucket seat n: a low separate seat for one person (as in automobiles and airplanes)

bucket shop n 1: a saloon in which liquor was formerly sold from or dispensed in open containers (as buckets or pichers) 2 a:

an establishment in which security and commodity options and uncompleted purchases and sales at trivial margins are handled like

bets b: a dishonest brokerage house; esp: one that fleeces customers by failing to execute orders on margin in anticipation of

market fluctuations adverse to their interest

buck-eye \bək-ī/ n 1: a shrub or tree (genus *Aesculus*) of the horse-chestnut family; also: its large nutlike seed 2 cap: a

native or resident of Ohio — used as a nickname

buck fever n: nervous excitement of an inexperienced hunter at the sight of game

'buck-le \bək-əl/ n [ME *bocle*, fr. MF, boss of a shield, buckle, fr. L *buccula*, dim. of *bucca* cheek — more at POKK] 2 a:

fastening for two loose ends that is attached to one and holds the other by a catch 2: an ornamental device that suggests a buckle

3 *archaic*: a crisp curl

2'buckle vb buck-led; buck-ling \bək-(ə-)lɪŋ/ vt 1: to fasten with a buckle 2: to prepare with vigor <buckled himself to the

task> 3: to cause to bend, give way, or crumple ~ vi 1: to apply oneself with vigor <~s down to the job> 2: to bend, heave, warp, or kink usu. under the influence of some external

agency <cornstalk buckling in the high wind> 3: COLLAPSE <the supports buckled under the strain> 4: to give way: YIELD <one

who does not ~ under pressure>

'buckle n: a product of buckling

'buck-ler \bək-lɪr/ n [ME *bocler*, fr. OF, shield with a boss, fr. *bocle*] 1 a: a small round shield held by a handle at arm's length b: a shield worn on the left arm 2: one that shields and protects

2'buckler vt: to shield or defend with a buckler

bucko \bək-(ə-)lɪr/ n, pl buck-oes 1: one who is domineering and bullying: SWAGGERER 2 chiefly Irish: young fellow: LAD

buck passer n ['buck]: a person who habitually passes the buck — *buck-pass-ing* \bək-pas-ɪŋ/ n

'buck-ram \bək-rəm/ n [ME *bukeram*, fr. OF *boquerant*, fr. OProv *bocaran*, fr. *Bokhara*, city of central Asia] 1: a stiff-

finished heavily sized fabric of cotton or linen used for interlinings in garments, for stiffening in millinery, and in bookbinding 2

*archaic*: STIFFNESS, RIGIDITY

2'buckram adj: suggesting buckram esp. in stiffness or formality

'buckram vt 1: to give strength or stiffness to (as with buckram)

2 *archaic*: to make pretentious

Bucks abbr Buckinghamshire

buck-saw \bək-sō/ n: a saw set in a usu. H-shaped frame that is used for sawing wood

buck-shee \bək-ˈʃhē/ n [Hindi *bakhsī*] 1 Brit: something extra obtained free; esp: extra rations 2 Brit: WINDFALL, GRATUITY

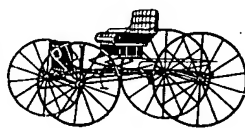
buck-shot \bək-ˈʃʃt/ n: a coarse lead shot

buck-skin \-ˈskɪn/ n 1 a: the skin of a buck b: a soft pliable usu. suede-finished leather 2 a pl: buckskin breeches b *archaic*:

a person dressed in buckskin; esp: an early American back-woodsman 3: a horse of a light yellowish dun color usu. with

dark mane and tail — *buckskin* adj

buck-tail \-ˈtāl/ n: an angler's lure made typically of hairs from the tail of a deer



buckboard



brussels sprouts

make brutal, y and disease> rs of war> —

us stupid, lit., of or relating NIMATE la 3 instinct: as a y reason <~ being of un-

short and ~ <~ gluttony> ack of under-

nash the teeth grinding the

waked from and is killed

-logy] 1: a moss life or

onia: akin to g vines of the

a about \* kitten or further a back ā bake ü cot, cart  
au out ch chin e less ē easy g gift i trip i life  
j joke ŋ sing ō flow ó flaw oi coin th thin th this  
ü loot ú foot y yet yū few yū furious zh vision